

Pollen studies in the genus *Viola* (Violaceae) from Iran

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Abstract – Pollen morphology of 17 species of *Viola* representing five sections, *Melanium*, *Plagiostigma*, '*Spathulidium*' ined., *Sclerosium*, and *Viola*, was studied using light and scanning electron microscope. Pollen grains were usually symmetrical, tetrazonocolporate to pentazonocolporate in section *Melanium* and trizonocolporate to tetrazonocolporate in the other four sections. Pollen shape was circular to subtriangular, tetragonal or pentagonal in polar view and prolate to oblate, spheroidal or pyramidal in equatorial view. Exine ornamentation was granulate, psilate and mostly perforate. The psilate type was only observed in *V. modesta*. We found heteromorphy in aperture number in *V. caspia* of section *Viola*, *V. occulta* of section *Melanium* and *V. behboudiana* of section *Sclerosium*, which corroborates their higher ploidy than in related species (octoploid versus tetraploid).

Key words: Exine, morphology, ornamentation, palynology, pollen, *Viola*

Introduction

Viola L. is the largest genus of Violaceae, with ca 600 species and is the only one widely distributed in the Northern Hemisphere (BALLARD et al. 1999). Infrageneric classification has varied, but recent phylogenetic analysis indicates that the genus can be subdivided into two subgenera and 16 sections worldwide (YOUSEFI et al. 2012). Five sections belonging to subgenus *Viola* are present in Iran: section *Melanium* Ging., section *Plagiostigma* Godr., section *Viola*, section *Sclerosium* W. Becker, and the undescribed section '*Spathulidium*' ined. The three first sections are widespread in the Northern Hemisphere and together comprise a few hundred species, while the two last sections are restricted to parts of southwestern Asia with fewer than a dozen species. The sections are ancient allotetraploid except for section '*Spathulidium*' which is inferred to be allooctoploid (MARCUSSEN et al. 2011). Recently, molecular phylogenetic analyses of sect. *Melanium* (including 25 species) were performed using ITS sequences and ISSR markers (YOCKTENG et al. 2003); they have shown

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that sect. *Melanium* forms a derived and monophyletic group. Section *Melanium*, the pansies, is represented in Iran by five annual-ephemeral species, *V. arvensis*, *V. kitaibeliana*, *V. modesta*, *V. occulta*, and *V. tricolor*. The only species of section *Plagiostigma* occurring in Iran, *V. somchetica*, belongs to subsect. *Patellares* (Boiss.) Rouy and Foucaud. Section *Viola* is restricted to northernmost Iran, where it occurs with six species, *V. alba* (subsp. *alba*), *V. odorata*, and *V. sintenisii* in subsect. *Viola* and *V. caspia*, *V. reichenbachiana*, and *V. rupestris* in subsect. *Rostratae*. Section *Sclerosium* is distributed in the South of Iran with three putative species, *V. behboudiana*, *V. cinerea* and *V. stocksii*. Section '*Spathulidium*' ined. refers to a small group of three central Asian species, in Iran represented by *V. spathulata* and *V. pachyrrhiza*; *V. maymanica* occurs in northern Afghanistan (SCHMIDT 1992). 17 species of *Viola* are distributed in different parts of Iran, mainly in the north. Of these, only *V. spathulata* is endemic to the territory (Elbrus mountains), but another five species are endemic to the region.

The delimitation of *Viola* species within sections and subsections can be problematic in many regions of the world, owing partly to phenotypic plasticity, partly to hybridisation and polyploidy, and partly to the few morphological characters separating taxa. Introgression appears, however, to be rare and restricted to certain groups (VALENTINE 1962, KRAHULKOVÁ et al. 1996, MARCUSSEN et al. 2001, HILDEBRANDT et al. 2006).

Pollen morphology of Violaceae in general has been examined by some authors (ERDTMAN 1952, PETTET 1964, WALKER and DOYLE 1975). A few additional studies have been published on pollen morphology in *Viola*. GORB (1994) studied 12 Ukrainian species of *Viola* sect. *Melanium* (including *V. arvensis*, *V. kitaibeliana*, and *V. tricolor*) using both light and scanning electron microscopy. PERVEEN and QAISER (2009) examined five *Viola* species (including *V. stocksii*) from Pakistan. DINÇ (2009) studied two species of subsection *Viola* (*V. sandrasea*, *V. kizildaghensis*) endemic to Turkey. Pollen heteromorphism is defined as the production by the same plant of pollen grains differing in aperture number (TILL-BOTTRAUD et al. 1995). This phenomenon occurs in over 30% of angiosperm species (MIGNOT 1994). In *Viola* pollen heteromorphism occurs in about a third of the studied species and appears to be associated with polyploidy (NADOT et al. 2000).

The main aim of this study is to provide a descriptive investigation of pollen grains in the genus *Viola* and also of pollen heteromorphism in the species distributed in Iran, using light and scanning electron microscopy. In light of the nomenclatural confusion and paucity of morphological characters for discrimination of *Viola* taxa in Iran (YOUSEFI et al. 2012), pollen morphology and heteromorphism may provide new characters useful for the delimitation of these taxa. In this study, pollen morphology of 13 species of the genus *Viola* with the exception of *V. arvensis*, *V. kitaibeliana*, *V. tricolor* and *V. stocksii* are described for the first time.

Material and methods

Pollen of 24 taxa representing the 17 species of *Viola* occurring in Iran was studied by means of light microscopy (LM) and scanning electron microscopy (SEM). Pollen samples were obtained from herbarium specimens collected the same year. In order to ensure the constancy of pollen characters among different populations of a certain species, 2–3 specimens per population were included in the analysis; otherwise each species was represented by only one population. The voucher specimens were deposited in Guilan University Her-

barium. A list of the voucher specimens is given in table 1. Pollen grains were stained with basic-fuchsin, then mounted in glycerin jelly and prepared for LM observation. Some measurements i.e. polar axis (P), equatorial axis (E), colpus length and exine thickness were made by LM (Nikon microscope model Optiphot-Z optic) for 30 pollen grains under a magnification of 1000x. For analysing pollen heteromorphism, the number of apertures were counted for 100 pollen grains, then the percentage of each aperture-class –three, four and five– were calculated and the percentage lower than 95 was reported as heteromorphic pollen (MIGNOT 1994).

For SEM observations, pollen grains soaked in absolute ethanol were pipetted directly on to 12.5 mm diameter stubs and air-dried at room temperature; they were then coated in a sputter coater with approximately 25 nm of Gold-Palladium. The micrographs were ob-

Tab. 1. Collection data of *Viola* species examined in the present. Species endemic to Iran are indicated by an asterisk (*).

Species	Sect. / Subsect.	Collection data
<i>V. arvensis</i> Murray	<i>Melanium</i> Ging.	Iran: Mazandaran, Javaherdeh, Jirkoh, May 1999, Naderifar, GUH-13682
<i>V. kitaibeliana</i> Schult.		Iran: Mazandaran, East slope of Damavand, above Malar, June 2000, Moosavi & Mozafarian, TARI-33184
<i>V. modesta</i> Fenzl		Iran: Kohgiluyeh-Boyer Ahmad, Nourabad to Yasuj road, 30 km to Yasuj, May 2009, Yousefi, GUH-13263-1
<i>V. occulta</i> Lehm.		Iran: Guilan, Deylaman, May 2009, Yousefi & Saeidi, GUH-13683
<i>V. tricolor</i> L.		Iran: Guilan, Rudbar, April 2009, Ghahremani, GUH-13663
<i>V. somchetica</i> C. Koch	<i>Plagiostigma</i> Godr./ <i>Estolonosae</i> Kupffer	Iran: Ardebil, Almas road, April 2009, Yousefi & Shahi, GUH-13674
<i>V. behboudiana</i> Rech. F. et Esfand.	<i>Sclerosium</i>	Iran: Baluchestan, Hudar, Ghasre ghand, 380 m, Foroughi, TARI-10755
<i>V. cineræ</i> Boiss.		Iran: Bushehr, 2–3 km of NE Khormoj, 150 m, Mozafarian, TARI- 17141
<i>V. stocksii</i> Boiss.		Iran: Hormozgan, Bandar-Abas, 20–30 km of W Rudan, 200 m, Wendelbo & Foroughi, TARI-15630
<i>V. spathulata</i> Willd.*	« <i>Spathulidium</i> » ined.	Iran: Mazandaran, Chalous-Karaj road, Duzdbon, April 1977, Ghahremani & Aghoostin, TUH-11252
<i>V. pachyrrhiza</i> Boiss. et Hohen.		Iran: Lorestan, Aligudarz road to Shoolabad, 2400 m, Runemark & Lazari, TARI-26228
<i>V. caspia</i> (Rupr.) Freyn 1	<i>Viola</i> <i>Rostratae</i> (Kupffer) W. Becker	Iran, Guilan., Lahijan, Sheitan koh, March 2008, Tabarestani, GUH-13634
<i>V. caspia</i> (Rupr.) Freyn 2		Iran: Guilan, Masouleh, Yousefi, GUH-4369
<i>V. caspia</i> (Rupr.) Freyn 3		Iran: Guilan, Lahijan, Sheitan koh, March 2007, Yousefi, GUH-13681
<i>V. reichenbachiana</i> Jord. ex Bor.		Iran, Guilan, Asalem to Khalkhal road, March 2009, Yousefi & Asaadi, GUH-13651

Tab. 1. – continued

Species	Sect. / Subsect.	Collection data
<i>V. rupestris</i> F. W. Schmidt		Iran: Ardebil, Almas road, April 2009, Yousefi & Asaadi, GUH-13672
<i>V. alba</i> Bess. subsp. <i>alba</i> 1	<i>Viola/Viola</i>	Iran: Guilan, Masouleh, March 2008, Royan, GUH-13266
<i>V. alba</i> Bess. subsp. <i>alba</i> 2		Iran: Guilan, Tootkabun, April 2009, Yousefi & Saeidi, GUH-13684
<i>V. alba</i> Bess. subsp. <i>alba</i> 3		Iran: Guilan, Lahijan, Sheitan koh, 2008, Yousefi, GUH-13271
<i>V. odorata</i> L. 1		Iran: Guilan, mountains of Masouleh, March 2008, Yousefi & Saeidi, GUH-13665
<i>V. odorata</i> L. 2		Iran: Guilan, Masouleh road, March 2007, Yousefi & Saeidi, GUH-13481-2
<i>V. sintenisii</i> W. Becker 1		Iran: Guilan, Lahijan, Sheitan koh, February 2008, Yousefi, GUH-13675
<i>V. sintenisii</i> W. Becker 2		Iran: Guilan, Lakan, February 2008, Yousefi, GUH-13281
<i>V. sintenisii</i> W. Becker 3		Iran: Guilan, Lashtenesha, the rice field, GUH-13296-2

tained from a LEO1430 VP (England) at an accelerating voltage of 10–15 kV under magnifications of 2040× to 11190×. Pollen terminology according to WALKER and DOYLE (1975), PUNT (2007) and HALBRITTER et al. (2008) has been followed.

Results

General pollen characters of the genus *Viola*

The pollen grains were shed in monads, symmetrical, tetrazonocolporate to pentazonocolporate in section *Melanium* and trizonocolporate to tetrazonocolporate in the other four sections (Figs. 1–2, Tab. 2). The apertures were colporate, equatorially elongated and reached the poles of the pollen with rounded or acute ends.

The smallest pollen grains belonged to *V. spathulata* ($P = 29 \mu\text{m}$, $E = 26.08 \mu\text{m}$) and the largest ones to *V. arvensis* ($P = 69.09 \mu\text{m}$, $E = 68.23 \mu\text{m}$). The P/E ratio varied between 0.94 and 1.18 (Tab. 2). The exine thickness varied between $0.6 \mu\text{m}$ (*V. spathulata*) and $1 \mu\text{m}$ (*V. occulta*).

Exine ornamentation proved to be a valuable taxonomic character in *Viola*. It varies from perforate, granulate, to psilate (Tab. 2). Among these patterns the simple psilate type represented by *V. modesta* is very characteristic.

Section *Melanium* (Figs. 1A–G, 3A–G)

Section *Melanium* is a morphologically well-defined group of about 125 species. Of these, five species are distributed in Iran, mostly in the north. Members of this section have

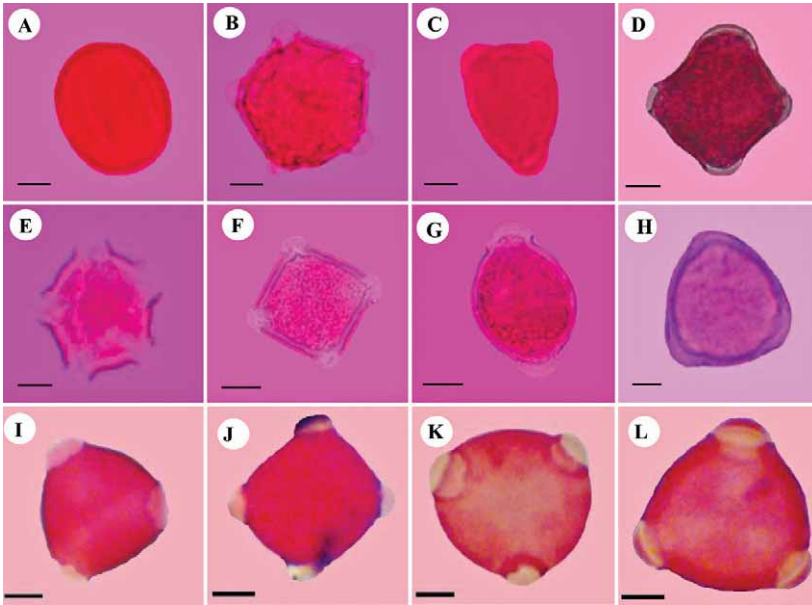


Fig. 1. Micrographs of pollen grains in *Viola arvensis* (A–B), *V. kitaibeliana* (C), *V. modesta* (D), *V. occulta* (E), *V. tricolor* (F–G), *V. somchetica* (H), *V. behboudiana* (I–J), *V. cinerea* (K), *V. stocksii* (L). Scale bar: 10 μ m.

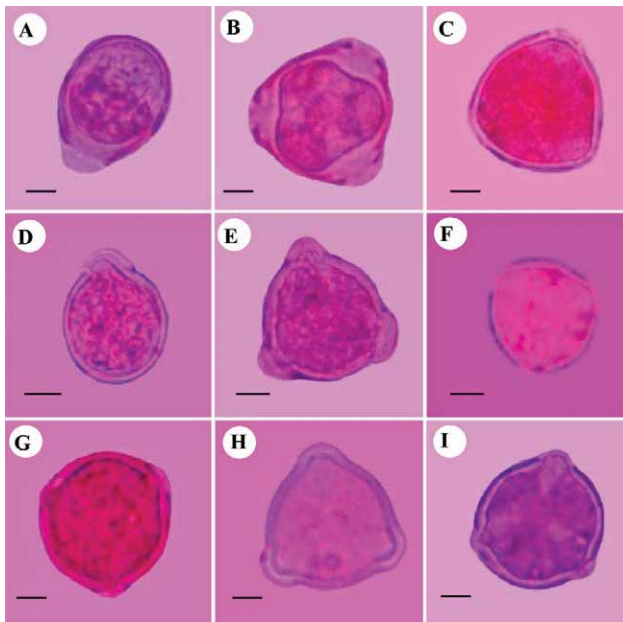


Fig. 2. Micrographs of pollen grains in *Viola pachyrrhiza* (A), *V. spatulata* (B), *V. caspia* (C), *V. reichenbachiana* (D–E), *V. rupestris* (F), *V. alba* (G), *V. odorata* (H), *V. sintenisii* (I). Scale bar: 10 μ m.

Tab. 2. Characteristic features of the investigated pollen in species of *Viola*. Abbreviations : P– polar axis (μm); E– equatorial axis (μm); M– mean value; SD – standard deviation; Eo – exine ornamentation; Pe: perforate, Gr: granulate, Ps: psilate.

Species	Sect. / Subject.	E (μm)		P (μm)		P	Eo
		Min (M ± SD)	Max	Min (M ± SD)	Max		
<i>V. arvensis</i>	<i>Melanium</i>	60.0 (68.2 ± 5.1)	85.0	62.5 (69.1 ± 3.7)	77.5	1/01	Pe-Gr
<i>V. kitaibeliana</i>		42.5 (45.6 ± 1.8)	47.5	30.0 (43.2 ± 7.9)	55.0	0/94	–
<i>V. modesta</i>		27.5 (34.8 ± 4.8)	40.0	40.0 (41.1 ± 3.2)	47.5	1/18	Ps
<i>V. occulta</i>		52.5 (60.0 ± 4.7)	67.5	55.0 (63.3 ± 4.0)	70.0	1/05	Pe-sub Ps
<i>V. tricolor</i>		50.0 (52.6 ± 2.1)	57.5	45.0 (54.9 ± 3.6)	62.5	1/04	Gr
<i>V. somchetica</i>	<i>Plagiostigma</i>	25.0 (29.0 ± 1.5)	32.5	27.5 (31.9 ± 2.0)	35.0	1/1	Pe- sub Ps
<i>V. behboudiana</i>	<i>Sclerosium</i>	29.4 (30.8 ± 1.1)	32.5	25.9 (29.5 ± 1.6)	31.7	0/95	Pe (fine)
<i>V. cinerae</i>		32.5 (33.9 ± 1.0)	35.7	33.0 (38.2 ± 2.0)	40.0	1/14	Pe (fine and large)
<i>V. stocksii</i>		38.2 (41.1 ± 1.7)	42.0	42.5 (44.0 ± 1.2)	45.0	1/07	Pe (fine)
<i>V. pachyrrhiza</i>	<i>Spathulidium</i>	23.0 (26.1 ± 1.7)	31.0	26.0 (29.0 ± 1.9)	34.0	1/11	Pe- Large
<i>V. spathulata</i>		22.5 (26.1 ± 1.7)	30.0	25.0 (29.0 ± 1.9)	32.5	1/11	Gr
<i>V. caspia</i> 1	<i>Viola.Rostratae</i>	32.5 (36.4 ± 1.7)	37.5	32.5 (36.5 ± 1.8)	40.0	1/00	(micro) Gr
<i>V. caspia</i> 2		27.5 (31.3 ± 2.2)	37.5	27.5 (33.5 ± 2.9)	37.5	1/07	Gr
<i>V. caspia</i> 3		40.0 (34.4 ± 3.6)	30.0	40.0 (35.5 ± 2.9)	32.5	1/03	Gr
<i>V. reichenbachiana</i>		27.5 (32.8 ± 3.7)	37.5	27.5 (32.6 ± 3.5)	37.5	0/9	Pe- sub Gr
<i>V. rupestris</i>		22.5 (25.3 ± 1.5)	30.0	27.5 (29.1 ± 1.6)	32.5	1/14	(micro) Gr
<i>V. alba</i> subsp. <i>alba</i> 1	<i>Viola.Viola</i>	25.0 (28.1 ± 1.9)	32.5	30.0 (31.6 ± 1.4)	35.0	1/12	Pe-sub Ps
<i>V. alba</i> subsp. <i>alba</i> 2		25.0 (30.3 ± 2.6)	35.0	27.5 (32.3 ± 3.0)	37.5	1/06	Pe- Gr
<i>V. alba</i> subsp. <i>alba</i> 3		25.0 (24.5 ± 1.1)	27.5	25.0 (28.8 ± 3.6)	30.0	1/17	Pe-sub Ps
<i>V. odorata</i> 1		25.0 (27.9 ± 2.6)	35.0	25.0 (29.5 ± 2.6)	35.0	1/05	Gr
<i>V. odorata</i> 2		25.0 (26.7 ± 1.3)	30.0	25.0 (31.6 ± 1.7)	35.0	1/18	(micro) Pe-Gr
<i>V. sintenisii</i> 1		25.0 (26.4 ± 2.0)	32.5	25.0 (30.3 ± 2.4)	35.0	1/14	Pe-Gr
<i>V. sintenisii</i> 2		22.5 (26.9 ± 3.7)	32.5	25.0 (30.6 ± 4.1)	37.5	1/13	Pe- Gr
<i>V. sintenisii</i> 3		25.0 (29.4 ± 3.7)	35.0	30.0 (33.8 ± 3.7)	40.0	1/14	Pe- Gr

four to five apertures, while a more frequent aperture number in members of other sections is three. The pollen type of *V. arvensis* is perforate–granulate (Figs. 3A–B).

At species level, *V. kitaibeliana* with pyramidal shape in equatorial view (Fig. 1C) can be distinguished from the other species, which are prolate to spheroidal in shape.

Exine ornamentation does not provide valuable characters in delimitation of section *Melanium* from other sections, but it is useful for species recognition within this section. For instance, two morphologically close species, *V. tricolor* and *V. modesta*, have different types of exine sculpturing, i.e. granulate and psilate, respectively (Figs. 3C, F–G). Furthermore, *V. arvensis*, which exhibits a perforate to granulate exine, can be separated from its relative *V. occulta*, which has perforate to subpsilate exine (Figs. 3D–E).

Section *Plagiostigma* (Figs. 1H, 3H–I)

Viola somchetica is the only Iranian species of this section and its pollen grain in equatorial view and exine ornamentation is prolate–spheroidal (Fig. 1H) and perforate–subpsilate (Figs. 3H–I), respectively. From a palynological point of view, this species does not show any obvious morphological apomorphies.

Section *Sclerosium* (Figs. 1I–L, 3J–L, 4A–C)

Viola behboudiana is easily separated from the other Iranian species of the section by two palynological characters: the shape of pollen grains, which is oblate–spheroidal in *V. behboudiana* (Figs. 1I–J) and prolate–spheroidal in *V. cinerea* and *V. stocksii* (Figs. 1K–L),

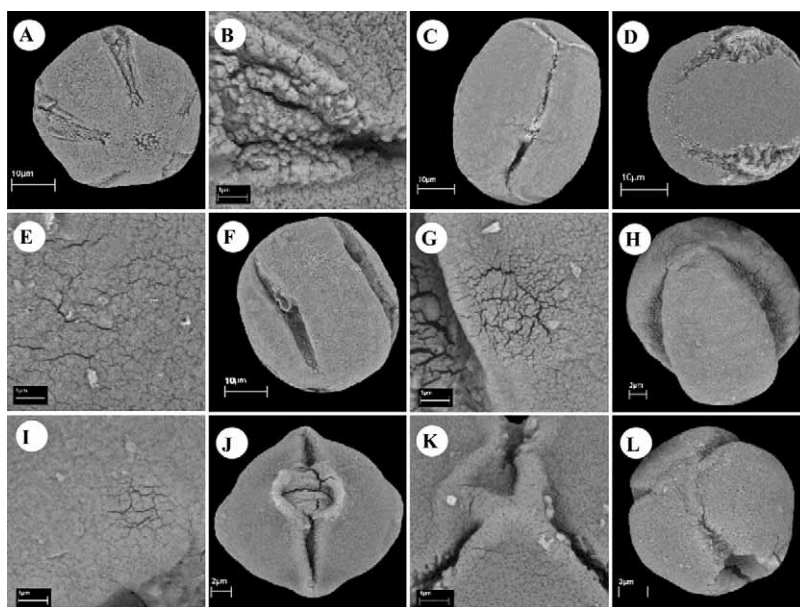


Fig. 3. SEM micrographs of pollen grains in *Viola arvensis* (A–B), *V. modesta* (C), *V. occulta* (D–E), *V. tricolor* (F–G), *V. somchetica* (H–I), *V. behboudiana* (J–K), *V. cinerea* (L).

and the presence of pollen heteromorphism in *V. behboudiana*. All of the three species have perforate to perforate–subpsilate exine ornamentation (Figs. 3J–L, 4A–C).

Section '*Spathulidium*' ined. (Figs. 2A–B, 4D–F)

The two Iranian species of Section *Spathulidium* are morphologically similar but can be easily separated using palynological characters: *V. pachyrrhiza* has perforate exine (Fig. 4D), whilst *V. spathulata* has granulate exine ornamentation (Figs. 4E–F). Pollen morphology shows a basic similarity between section *Spathulidium* and section *Plagiostigma*; however these two sections can be differentiated according to exine ornamentation (Tab. 2).

Section *Viola*

No palynological characters separate this section from the others.

Subsection *Rostratae* (Figs. 2C–F, 4G–L, 5A)

This subsection has three species in Iran, *V. caspia*, *V. reichenbachiana* and *V. rupestris*. The morphological recognition of the first two species is very problematic due to the variable morphological characters; however palynological studies provide useful characters for delimiting these taxa. Pollen grains of *V. caspia* are prolate–spheroidal to subprolate in equatorial view (Fig. 2C) with a granulate exine (Figs. 4G–J), while *V. reichenbachiana* has oblate–spheroidal pollen grains (Figs. 2D–E) with a perforate–subgranulate exine (Fig. 4K). Furthermore, *V. caspia* shows pollen heteromorphism, in which the dominant pollen shape has three apertures and 6 % of pollen grains have four apertures. The characteristics of pollen sculpturing of *V. rupestris* are similar to *V. caspia* (Figs. 2F, 4L, 5A), but the P/E ratio of *V. rupestris* is larger than *V. caspia* (Tab. 2).

Subsection *Viola* (Figs. 2G–I, 5B–I)

This subsection has three species in Iran, *V. alba*, *V. sintenisii*, and *V. odorata*. Whereas the latter can be easily recognised using morphological characters, i.e. broad stipules and high number of leaf crenulae, the division between two first species is still problematic. The shape in equatorial view of three species is prolate–spheroidal to subprolate (Figs. 2G–I). Pollen characteristics are also variable between the taxa, particularly, the exine ornamentation shows that *V. alba* has perforate to subpsilate and perforate to granulate exine (Figs. 5B–C), *V. sintenisii* has perforate–granulate (Fig. 5H–I). *Viola odorata* has perforate to subpsilate and perforate to granulate exine (Figs. 5D–G).

Discussion

The exine sculpturing of the pollen grains, their size, shape and aperture numbers can be of taxonomic value in the studied taxa. The pollen size variability is a diagnostic value between some of the taxa within the same section, for example, between *V. arvensis* and *V. kitaibeliana* (sect. *Melanium*), *V. modesta* and *V. occulta* (sect. *Melanium*), *V. cinerea* and *V. stocksii* (sect. *Sclerosium*).

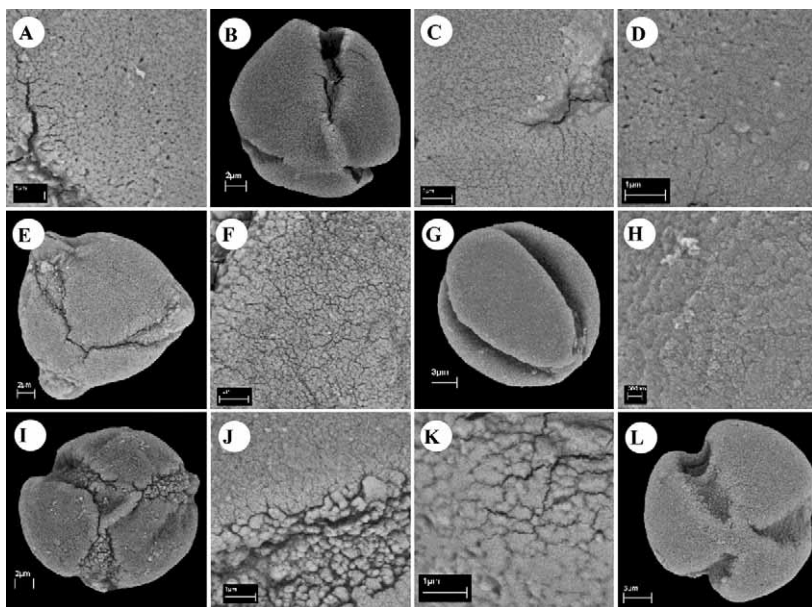


Fig. 4. SEM micrographs of pollen grains in *Viola cinerea* (A), *V. stocksii* (B–C), *V. pachyrrhiza* (D), *V. spathulata* (E–F), *V. caspia* (G–J), *V. reichenbachiana* (K), *V. rupestris* (L).

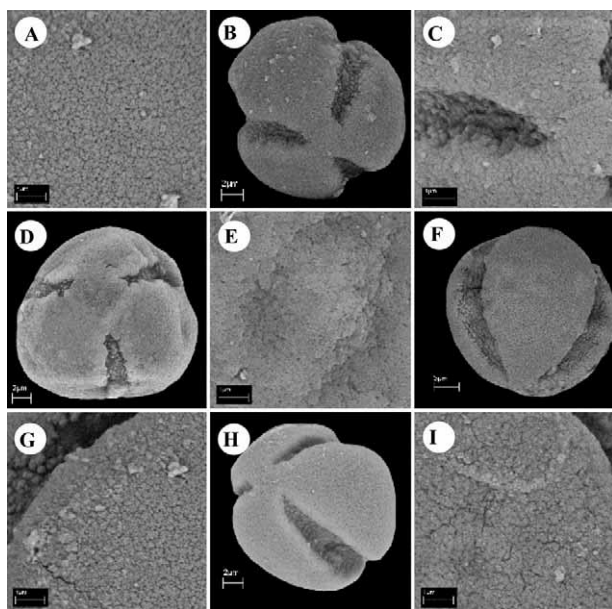


Fig. 5. SEM micrographs of pollen grains in *Viola rupestris* (A), *V. alba* (B–C), *V. odorata* (D–G), *V. sintenisii* (H–I).

Tab. 3. Characteristic features of the investigated pollen in species of *Viola*. M – mean value; SD – standard deviation

Species	Sect. / Subsect.	Colpate (μm)		Exin thickness (μm)	Shape (equatorial view)	Shape (polar view)
		Min (M ± SD)	Max			
<i>V. arvensis</i>	<i>Melanium</i>	52.5 (60.4 ± 4.8)	75.0	2.2 ± 0.2	Prolate spheroidala	Pentagonal
<i>V. kitaibeliana</i>		35.0 (40.0 ± 2.8)	42.5	2.1 ± 0.2	Pyramidal	Tetragonal
<i>V. modesta</i>		20.0 (24.5 ± 2.2)	27.5	2.3 ± 0.2	Prolate spheroidala	Tetragonal
<i>V. occulta</i>		47.5 (53.9 ± 4.2)	62.5	2.5 ± 0.1	Prolate spheroidala	Tetragonal
<i>V. tricolor</i>		42.5 (46.0 ± 1.7)	47.5	2.3 ± 0.2	Prolate spheroidala	Tetragonal
<i>V. somchetica</i>	<i>Plagiostigma</i>	20.0 (22.8 ± 1.6)	25.0	1.6 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. behboudiana</i>	<i>Sclerosium</i>	22.0 (23.3 ± 1.4)	25.0	1.8 ± 0.1	Oblate spheroidal	Circular-Subtriangular
<i>V. cinerae</i>		16.5 (17.6 ± 0.8)	18.7	1.2 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. stocksii</i>		19.7 (19.8 ± 0.1)	20.0	2.7 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. pachyrrhiza</i>	<i>Spathulidium</i>	20.0 (22.3 ± 1.6)	26.0	1.4 ± 0.1	Prolate spheroidala	Circular-Subtriangular
<i>V. spathulata</i>		20.0 (22.3 ± 1.6)	25.0	1.4 ± 0.1	Prolate spheroidala	Circular-Subtriangular
<i>V. caspia</i> 1	<i>Viola.Rostratae</i>	22.5 (27.9 ± 2.7)	32.5	1.8 ± 0.1	Prolate spheroidala	Circular-Subtriangular
<i>V. caspia</i> 2		30.0 (31.5 ± 1.2)	33.8	1.7 ± 0.1	Spheroidal	Circular-Subtriangular
<i>V. caspia</i> 3		30.0 (26.8 ± 2.7)	22.5	2.0 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. reichenbachiana</i>		22.5 (28.0 ± 3.7)	35.0	2.4 ± 0.2	Oblate spheroidal	Circular-Subtriangular
<i>V. rupestris</i>		17.5 (21.6 ± 1.8)	25.0	2.2 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. alba</i> subsp. <i>alba</i> 1	<i>Viola.Viola</i>	17.5 (21.8 ± 0.2)	25.0	2.0 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. alba</i> subsp. <i>alba</i> 2		22.5 (24.6 ± 2.5)	30.0	2.2 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. alba</i> subsp. <i>alba</i> 3		20.0 (22.5 ± 1.8)	25.0	2.0 ± 0.6	Prolate spheroidala	Circular-Subtriangular
<i>V. odorata</i> 1		17.5 (20.8 ± 1.6)	22.5	2.0 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. odorata</i> 2		20.0 (21.2 ± 1.2)	20.0	2.2 ± 0.2	Subprolatea	Circular-Subtriangular
<i>V. sintenisii</i> 1		20.0 (21.0 ± 1.2)	22.5	1.8 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. sintenisii</i> 2		22.5 (24.4 ± 2.1)	27.5	1.9 ± 0.2	Prolate spheroidala	Circular-Subtriangular
<i>V. sintenisii</i> 3		25.0 (27.5 ± 1.8)	30.0	1.5 ± 0.1	Prolate spheroidala	Circular-Subtriangular

Tab. 4. The number of aperture and pollen heteromorphism in the investigated species of *Viola*.

Species	Sect. . Subsect.	Number of apertures (3 + 4 + 5)	Percent of most abundant pollen morph (%)	Hetero-morphism (<95 %)	Predominant pollen morph
<i>V. arvensis</i>	<i>Melanium</i>	0 + 2 + 98	98	–	5 zonocolporate
<i>V. kitaibeliana</i>		0 + 97 + 3	97	–	4 zonocolporate
<i>V. modesta</i>		0 + 96 + 4	96	–	4 zonocolporate
<i>V. occulta</i>		0 + 85 + 15	85	+	4 zonocolporate
<i>V. tricolor</i>		0 + 97 + 3	97	–	4 zonocolporate
<i>V. somchetica</i>	<i>Plagiostigma</i>	100 + 0 + 0	100	–	3 zonocolporate
<i>V. behboudiana</i>	<i>Sclerosium</i>	92 + 8 + 0	92	+	3 zonocolporate
<i>V. cinerae</i>		99 + 0 + 0	100	–	3 zonocolporate
<i>V. stocksii</i>		98 + 2 + 0	98	–	3 zonocolporate
<i>V. pachyrrhiza</i>		98 + 2 + 0	98	–	3 zonocolporate
<i>V. spathulata</i>	<i>Spathulidium</i>	98 + 2 + 0	98	–	3 zonocolporate
<i>V. caspia</i> 1	<i>Viola/Rostratae</i>	94 + 6 + 0	94	+	3 zonocolporate
<i>V. caspia</i> 2		97 + 3 + 0	97	–	3 zonocolporate
<i>V. reichenbachiana</i>		99 + 1 + 0	99	–	3 zonocolporate
<i>V. rupestris</i>		100 + 0 + 0	100	–	3 zonocolporate
<i>V. alba</i> subsp. <i>alba</i> 1	<i>Viola/Viola</i>	100 + 0 + 0	100	–	3 zonocolporate
<i>V. alba</i> subsp. <i>alba</i> 2		100 + 0 + 0	100	–	3 zonocolporate
<i>V. alba</i> subsp. <i>alba</i> 3		100 + 0 + 0	100	–	3 zonocolporate
<i>V. odorata</i> 1		100 + 0 + 0	100	–	3 zonocolporate
<i>V. odorata</i> 2		100 + 0 + 0	100	–	3 zonocolporate
<i>V. sintenisii</i> 1		100 + 0 + 0	100	–	3 zonocolporate
<i>V. sintenisii</i> 2		100 + 0 + 0	100	–	3 zonocolporate
<i>V. sintenisii</i> 3		100 + 0 + 0	100	–	3 zonocolporate

Pollen grains in polar view are variable within the sect. *Melanium* and the other sections (Tab. 2). A tetragonal to pentagonal shape in polar view is found in *V. arvensis*, *V. kitaibelliana*, *V. modesta*, *V. occulta* and *V. tricolor* (sect. *Melanium*), whilst circular-subtriangular is present in the other sections. The pollen shape of sect. *Melanium* was circular to sub-triangular, tetragonal or pentagonal in polar view and prolate to oblate, spheroidal or pyramidal in equatorial view according to the ratio of polar-equatorial axis (WALKER and DOYLE 1975).

According to the classification of pollen grains based on size (WALKER and DOYLE 1975), this section has large pollen grains (50–99 μm), while the other sections of *Viola* distributed in Iran have medium sized pollen grains (25–49 μm).

Pollen grains with six apertures were reported for *V. arvensis* from Ukraine (GORB 1994). The figures given in ERDTMAN (1952) show that *V. arvensis* has 4-colpate with a few 5-colpate grains appearing to be based on misidentification with *V. tricolor* since this type is found in *V. tricolor* and not *V. arvensis*. PETTET (1964) noted that the two very variable and often confused species, *V. tricolor* and *V. arvensis*, are readily separable on the basis of pollen assemblage.

Earlier studies suggested that section *Plagiostigma* is not closely related to section *Viola*, as previously assumed (CLAUSEN 1929, MARCUSSEN et al. 2012). Our palynological results for Iranian *V. stocksii* agree with findings for this species in Pakistan (PERVEEN and QAISER 2009).

Previously these species, i.e. *V. pachyrrhiza* and *V. spathulata* were included in section *Plagiostigma* subsection *Patellares* (as section *Nomimium* grex *Adnatae*; BECKER 1918). However, this lineage takes a completely different phylogenetic position and will require a section of its own (MARCUSSEN et al. 2010). Yousefi et al. (2012) demonstrated that *Viola somchetica* (section *Plagiostigma*) differs from *V. spathulata* (section *Spathulidium*) in the number of collenchyma layers at the corners of petiole cross sections (4 vs. 3) and the shape of peduncle cross sections (quadrangular vs. circular).

Morphological and phylogenetic evidence supports two subsections, subsection *Rostratae*, with aerial floriferous stems and explosive capsules, and subsection *Viola*, lacking aerial stems and with inexplusive capsules (BECKER 1925, MARCUSSEN and KARLSSON 2010). Anatomically, *V. caspia* can be separated from *V. reichenbachiana* by the presence of a pith region in root cross-sections and by the number of vascular bundles in stem cross sections (YOUSEFI et al. 2012).

Viola behboudiana (section *Sclerosium*), *V. caspia* (section *Viola*) and *V. occulta* (section *Melanium*) showed structural polymorphism. The number of apertures in the two first ones was 3–4, and 4–5 in the last one. All three species are high-polyploids. *Viola behboudiana* and *V. caspia* are both octoploids (MARCUSSEN and BORGES 2011), and this is probably the case also for *V. occulta* ($n = 10$ versus $n = 3, 4, 5, 7$ in its close tetraploid relatives; YOCKTENG et al. 2003). In the genus *Viola*, pollen heteromorphism is a direct result of polyploidy (NADOT et al. 2000).

Acknowledgements

We thank to Mr. A. Khodayari (University of Ardebil) for his assistance in electron microscopy. This research was supported by a grant 989–27 from the research council of the University of Guilan.

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